PTO/SB/01 (10-05)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

☑ Declaration Submitted With Initial Filing **OR** ☐ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	017191.0049
First Named Inventor	Michael J. Pugia
COMPLETE IF KNOWN	
Application Number	Unassigned
Filing Date	
Art Unit	Unknown
Examiner Name	Unknown

I hereby declare that:

Each inventor's residence, mailing address, and citizenship are as stated below next to their name

I believe the inventor(s) named below to be the original and first inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MONOCLONAL ANTIBODIES FOR DETECTION OF URINARY TRYPSIN INHIBITORS

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☑ was filed on (MM/DD/YYYY) 07/29/2004 as United States Application Number or PCT International Application Number PCT/US2004/024881 and was amended on (MM/DD/YYYY) 08/12/2005 (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

PTO/SB/01 (10-05)
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DECLARATION — Utility or Design Patent Application

Direct all correspondence to: ☐ The address associated with Customer Number: _____ OR ☑ Correspondence address below

Name
Bayer Healthcare LLC
Address
511 Benedict Avenue

City	State	ZIP
Tarrytown	New Jersey	10591

Country	Telephone	Email
USA	914-524-2684	kevin.stein.b@bayer.com

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Michael J.	Pugia

Inventor's Signature	Date

Residence: City	State	Country	Citizenship
Granger	Indiana	USA	USA

Mailing Address
14342 Taddington Drive

City	State	Zip	Country
Granger	Indiana	46530	USA

☑ Additional inventors or a legal representative are being named on the 2 supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.

PTO/SB/02A (09-04)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle (if any))	Family Name or Surname
Linda	Anderson-Mauser

Inventor's Signature _____ Date _____

Residence: City	State	Country	Citizenship
Elkhart	Indiana	USA	USA

60438 County Road 3

Mailing Address

City	State	Zip	Country
Elkhart	Indiana	46517	USA

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle (if any))	Family Name or Surname
Solomon H.	Murphy

Inventor's Signature Solomon H. Murphy Date 3/30/2006

Residence: City	State	Country	Citizenship
Spring	Texas	USA	USA

8900 Research Park Drive, Apt. 1710

Mailing Address

City	State	Zip	Country
Spring	Texas	77381	USA

Name of Additional Joint Inventor, if any: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle (if any))	Family Name or Surname
Ronald G.	Sommer

Inventor's Signature _____ Date _____

Residence: City	State	Country	Citizenship
Elkhart	Indiana	USA	USA

55745 Merle Street

Mailing Address

City	State	Zip	Country
Elkhart	Indiana	46514	USA

PTO/SB/02A (09-04)
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DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor
Given Name (first and middle (if any))	Family Name or Surname
Shannon	Gleason
Inventor's Signature	Date

Residence: City	State	Country	Citizenship
Jones	Michigan	USA	USA

14006 Carter Lake Street

Mailing Address

City	State	Zip	Country
Jones	Michigan	49061	USA

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor
Given Name (first and middle (if any))	Family Name or Surname
Inventor's Signature	Date

Residence: City	State	Country	Citizenship

Mailing Address

City	State	Zip	Country

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Given Name (first and middle (if any))	Family Name or Surname
Inventor's Signature	Date

Residence: City	State	Country	Citizenship

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PTO/SB/01 (10-05)
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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63)

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MONOCLONAL ANTIBODIES FOR DETECTION OF URINARY TRYPSIN INHIBITORS

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

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Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐
			☐	☐	☐

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DECLARATION — Utility or Design Patent Application

Direct all correspondence to: ☐ The address associated with Customer Number: _____ OR ☑ Correspondence address below

Name
Bayer Healthcare LLC
Address
511 Benedict Avenue

City	State	ZIP
Tarrytown	New Jersey	10591

Country	Telephone	Email
USA	914-524-2684	kevin.stein.b@bayer.com

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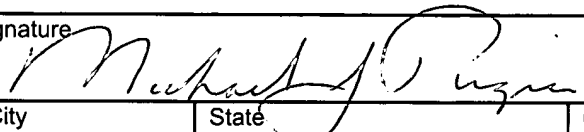
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
Michael J.	Pugia

Inventor's Signature:  Date: 3/29/06

Residence: City	State	Country	Citizenship
Granger	Indiana	USA	USA

Mailing Address
14342 Taddington Drive

City	State	Zip	Country
Granger	Indiana	46530	USA

☑ Additional inventors or a legal representative are being named on the 2 supplemental sheet(s) PTO/SB/02A or 02LR attached hereto.

PTO/SB/02A (09-04)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor		
Given Name (first and middle (if any))	Family Name or Surname		
Linda	Anderson-Mauser		
Inventor's Signature Linda Anderson-Mauser			Date March 29, 2006
Residence: City: Elkhart	State: Indiana	Country: USA	Citizenship: USA
Mailing Address: 60438 County Road 3			
City: Elkhart	State: Indiana	Zip: 46517	Country: USA

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor		
Given Name (first and middle (if any))	Family Name or Surname		
Solomon H.	Murphy		
Inventor's Signature			Date
Residence: City: South Bend	State: Indiana	Country: USA	Citizenship: USA
Mailing Address: 295 East Lasalle Avenue, Apt. 301a			
City: South Bend	State: Indiana	Zip: 46617	Country: USA

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor		
Given Name (first and middle (if any))	Family Name or Surname		
Ronald G.	Sommer		
Inventor's Signature Ronald G. Sommer			Date 03/28/06
Residence: City: Elkhart	State: Indiana	Country: USA	Citizenship: USA
Mailing Address: 55745 Merle Street			
City: Elkhart	State: Indiana	Zip: 46514	Country: USA

PTO/SB/02A (09-04)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor		
Given Name (first and middle (if any))	Family Name or Surname		
Shannon	Gleason		
Inventor's Signature: Shannon L Gleason	Date 28 MARCH 2006		
Jones Residence: City	Michigan State	USA Country	USA Citizenship
14006 Carter Lake Street Mailing Address			
Jones City	Michigan State	49061 Zip	USA Country

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor		
Given Name (first and middle (if any))	Family Name or Surname		
Inventor's Signature	Date		
Residence: City	State	Country	Citizenship
Mailing Address			
City	State	Zip	Country

Name of Additional Joint Inventor, if any:	☐ A petition has been filed for this unsigned inventor		
Given Name (first and middle (if any))	Family Name or Surname		
Inventor's Signature	Date		
Residence: City	State	Country	Citizenship
Mailing Address			
City	State	Zip	Country

PTO/SB/02B (09-04)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

DECLARATION – Supplemental Priority Data Sheet

Foreign applications:

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			☐	☐	☐
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PTO/SB/81 (01-06)
Approved for use through 12/31/08. OMB 0651-0035
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

POWER OF ATTORNEY and CORRESPONDENCE ADDRESS INDICATION FORM

Application Number	Unassigned
Filing Date	
First Named Inventor	Michael J. Pugia et al.
Title	Monoclonal Antibodies for Detect...
Art Unit	
Examiner Name	
Attorney Docket Number	017191.0049

I hereby revoke all previous powers of attorney given in the above-identified application.

I hereby appoint:

☐ Practitioners associated with the Customer Number:

OR

☒

Name; Registration Number	Name; Registration Number
Kevin Stein; 47,966	Harold N. Wells; 26,044
Chien Yuan; 48,056	S.Z. Szczepanski; 27,957
Rupa Sen; 42,139	Mary Jo Boldingh34,713
Mark Seka; 44,330	Katherine L. Tabor; 36,026
Andrew Klawitter; 26,557	Glen J. Gesicki; 55,863

as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith.

Please recognize or change the correspondence address for the above-identified application to:

☐ The address associated with the above-mentioned Customer Number:

OR

☐ The address associated with Customer Number:

OR

☒ Firm or Individual Name	Bayer Healthcare LLC				
Address	511 Benedict Avenue				
City	Tarrytown	State	New York	Zip	10591
Country	USA				
Telephone	914-524-2684	Email	Kevin.stein.b@bayer.com		

I am the:

☐ Applicant/inventor.

☒ Assignee of record of the entire interest. See 37 CFR 3.71.
Statement under 37 CFR 3.73(b) is enclosed. (Form PTO/SB/96)

SIGNATURE of Applicant or Assignee of Record

Signature		Date	04/03/06
Name	Kenneth F. Wobbekind	Telephone	914 524 2741
	Vice President, Associate General Counsel		
Title and Company	Bayer Healthcare LLC		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

PTO/SB/96 (12-05)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

STATEMENT UNDER 37 CFR 3.73(b)

Applicant/Patent Owner: Bayer Healthcare LLC

Application No./Patent No./Control No.: 60/511,835 Filed/Issue Date: October 16, 2003

Entitled: Monoclonal Antibodies for Detection of Urinary Trypsin Inhibitors

Bayer Healthcare LLC, a corporation
(Name of Assignee) (Type of Assignee: corporation, partnership, university, government agency, etc.)

states that it is:

1. ☑ the assignee of the entire right, title, and interest; or

2. ☐ an assignee of less than the entire right, title and interest
(The extent (by percentage) of its ownership interest is _____%)

in the patent application/patent identified above by virtue of either:

A. ☑ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 014388, Frame 0379, or a true copy of the original assignment is attached.

OR

B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet.

As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (*i.e.*, a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

Signature	April 3, 2006 Date
Kenneth F. Wobbekind Printed or Typed Name	914 524 2741 Telephone Number
Vice President, General Counsel and Assistant Secretary Title	

PATENT
017191.0049

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re U.S. Patent Application	Unassigned (related to PCT/US2004/024881)	Customer No.: 47670
Applicant:	Pugia et al.	
Serial No.:	Unassigned	
Filed:	Herewith	
For:	**Monoclonal Antibodies for Detection of Urinary Trypsin Inhibitors**	

I hereby certify that this correspondence is being deposited with the United Postal Service as first class mail in an envelope addressed to: Commissioner of Patents, P. O. Box 1450, Alexandria, VA, 22313-1450, on 4/6/06

Signature

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

1. I am one of the named inventors in the subject application. I received a Ph.D. degree in Chemistry from Texas Tech University and have been employed by Bayer Healthcare LLC and its predecessors since 1986. My present title is Director, New Products.

2. My purpose is to place on the record the composition used as immunogen to raise the monoclonal antibodies reported in the subject application. As stated on page 10 of the application, UTIs from renal patients were purified by SciPac Ltd. and used as the immunogen. Our intent was to raise monoclonal antibodies against purified uristatin.

3. In an amendment under PCT Article 34 submitted on August 12, 2005, the composition of the immunogen used to inoculate mice in the preparation of monoclonal antibodies was corrected. As filed, the composition (on page 10) was "15-20% 17 kDa, 50-55%, 35 kDa, and 25-30%, 60-80- kDa with some material in the 2 to 12 kDa range. It was replaced by "about > 85% of the material 17 kDa (uristatin), plus > 10% uristatin -1 or -2 and < 5% of bikunin (30.9 kDa) and no detectable AMBK, I-α-I, or P-α-I." This correction was required

when it was discovered that the composition of the immunogen actually used to inoculate mice had been incorrectly associated with the composition of purified UTI lot 20-120, rather than the composition of the UTI lot 157-90 actually used. The composition of UTI lot 157-90 was predominantly uristatin, which has a molecular weight of about 17 kDa, as will be shown in the accompanying documents. The composition of UTI lot 20-120 was reported also in Example 3 of the application. It contains substantial amounts of bikunin and only small amounts of uristatin.

Since the International Preliminary Examination Report objected to correction of the immunogen composition, the erroneous composition has been deleted in the accompanying preliminary amendment.

4. That UTI lot 157-90 was used as the immunogen is shown in the attached memorandum by Solomon Murphy of April 24, 2002, requesting that 15 mice be immunized with UTI lot 157-90.

5. Three lots of purified UTI were analyzed by Shannon F. Gleason in the attached notebook pages 84-85 dated September 9, 2002. All of the UTI lots were predominantly uristatin as the size of the blots indicate. Lane 4 is understood to represent lot 157-90. The computed results are not consistent with the size of the blots and are considered to be incorrect.

6. The composition of UTI lots 157-90 and 20-120 are shown from SDS-PAGE separations in the attached memorandum of July 7, 2003 from Shannon Gleason and Nancy C. Leszczynski, which reports tests done with several UTI lots. They state that UTI lots 157-90 and 124-111 were predominantly uristatin (≈17 kDa), while UTI lot 20-120 was predominantly bikunin (≈35 kDa). This is illustrated in Figure 1 where UTI lots 124-111 and 157-90 are seen to be similar, while UTI lot 20-120 is clearly different. Note that the results with non-reducing gels are reported, since the reducing gel changes the composition, as can be seen in Figure 2.

7. A later report from Nancy C. Leszczynski and Shannon Gleason, dated October 15, 2003, reported on page 4 that UTI lot 20-120 had 100% at 33 kDa molecular weight. UTI lot 157-90 had 53% at 18 kDa and 47% at 34 kDa, while UTI lot 124-111 had 97% at 16kDa and 3% at 32 kDa. These results for UTI lot 157-90 differed from the July 7, 2003 report.

8. In a review, dated November 12, 2003, of the results reported in paragraph 7, Ronald Sommer used an algorithm to interpret the SDS-PAGE results, as is shown in the accompanying copies of pages from Mr. Sommer's lab notebook. The non-reducing gel results were reported in

the October 15, 2003 memorandum as Figure 1 on page 6. One can conclude that UTI lot 157-90 was similar to that of UTI lot 124-111, but not to UTI lot 20-120, both lots 157-90 and 20-120 having a strong peak at 100, corresponding to the 17 kDa band, while UTI lot 20-120 did not have such a peak and instead had its strongest peak at about 160, corresponding to bikunin (35 kDa) rather than uristatin.

9. The report dated March 29, 2004 by Nancy C. Leszczynski notes that temperature affects the composition of the UTI lots, which show that more uristatin is present as temperature increases. Figure 1 compares UTI lots 20-120, 124-111, and 157-90 at three temperatures. It will be evident that lots 157-90 and 124-111 are similar in being substantially uristatin, while lot 20-120 is substantially bikunin, plus a higher molecular weight band.

10. I hereby declare that all statements made of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

3-29-06
Date

Michael J. Pugia, Ph.D.

INDEX

DESCRIPTION

EXP. BUCHANAN, MI / FEB. 2000

BAYER CORPORATION

SUBJECT

Bayer

Business Group
Diagnostics

Internal Memorandum

Research & Development
Laboratory

Date: April 24, 2002

Subject: Monoclonal Antibody Development to Urinary Trypsin Inhibitor

From: Solomon Murphy

To: G. Jackson

Cc: G. Craigo

Please immunize 15 mice with Urinary Trypsin Inhibitor for monoclonal antibody production. Immunize with 100ug/mouse.

The 15 mice consist of 5 Freund's , 5 GERBU and 5 RIBI.

Immunogen:

Urinary Trypsin Inhibitor
Lot No. 157-90
1ml @ 1mg/mL
Store @ 2-8 C

Thank you,

Solomon Murphy

SIGNED BY _____ DATE July 10 2002

WITNESSED AND UNDERSTOOD BY _____ DATE Sept 6, 2002

CROSS REFERENCES:

BAYER CORPORATION

SUBJECT _____

Western Blot of Plasma Samples

Scanned image was flipped so orientation is the same as the SDS-PAGE.

MagicMark standards in Lane 2 were used to estimate Molecular weights

Detail Report by Lane
072302pw
September 2, 2002

Lane 1 See Blue Plus 2

Band #	Rf	MgMk kDa	Peak RD	Trace RD x mm	Percent of Bands	Contour RD x mm²	Quantity	Band Name
1 - 2	0.127	216.03x	0.06	0.035	16.4			
1 - 3	0.220	108.44	0.12	0.177	83.6			

Lane 2 Magic Mark

Band #	Rf	MgMk kDa	Peak RD	Trace RD x mm	Percent of Bands	Contour RD x mm²	Quantity	Band Name
2 - 1	0.207	120.00*	0.51	0.238	6.6			
2 - 2	0.231	100.00*	0.45	0.190	5.3			
2 - 3	0.280	80.00*	0.34	0.198	5.5			
2 - 4	0.357	60.00*	0.62	0.592	16.4			
2 - 5	0.415	50.00*	0.44	0.446	12.3			
2 - 6	0.491	40.00*	0.59	0.599	16.6			
2 - 7	0.591	30.00*	0.46	0.469	13.0			
2 - 8	0.696	20.00*	0.65	0.884	24.4			

Lane 3 uRistatin - 117

Band #	Rf	MgMk kDa	Peak RD	Trace RD x mm	Percent of Bands	Contour RD x mm²	Quantity	Band Name
3 - 1	0.572	31.72	0.21	0.522	11.5			
3 - 2	0.744	16.56x	0.59	4.003	88.5			

SIGNED BY Shannon L. Glass **DATE** 9 Sept 2002

WITNESSED AND UNDERSTOOD BY _____ **DATE** 26 Oct 2002

CROSS REFERENCES:

BAYER CORPORATION

SUBJECT _____

Lane 4 Ueistatin-90

Band #	Rf	MgMk kDa	Peak RD	Trace RD x mm	Percent of Bands	Contour RD x mm²	Quantity	Band Name
4 - 1	0.348	62.15	0.29	0.717	13.1			
4 - 3	0.583	30.74	0.36	3.220	58.9			
4 - 6	0.738	17.00x	0.51	1.529	28.0			

Lane 5 Ueistatin-111

Band #	Rf	MgMk kDa	Peak RD	Trace RD x mm	Percent of Bands	Contour RD x mm²	Quantity	Band Name
5 - 3	0.608	28.11	0.34	3.435	57.6			
5 - 5	0.708	19.10x	0.40	0.481	8.1			
5 - 8	0.748	16.31x	0.51	2.053	34.4			

Lane 6 Plasma 04

Band #	Rf	MgMk kDa	Peak RD	Trace RD x mm	Percent of Bands	Contour RD x mm²	Quantity	Band Name
6 - 2	0.060	354.49x	0.14	0.320	13.1			
6 - 5	0.212	115.56	0.35	0.603	24.7			
6 - 6	0.268	84.51	0.40	0.962	39.4			
6 - 10	0.449	45.29	0.28	0.274	11.2			
6 - 11	0.490	40.19	0.24	0.285	11.7			

Lane 7 Plasma 30

Band #	Rf	MgMk kDa	Peak RD	Trace RD x mm	Percent of Bands	Contour RD x mm²	Quantity	Band Name
7 - 2	0.057	362.71x	0.13	0.542	12.1			
7 - 4	0.173	154.31x	0.34	0.483	10.8			
7 - 6	0.281	79.63	0.41	1.333	29.8			
7 - 8	0.427	48.27	0.34	0.618	13.8			
7 - 9	0.454	44.62	0.32	0.492	11.0			
7 - 10	0.482	41.18	0.31	0.622	13.9			
7 - 11	0.517	37.13	0.31	0.379	8.5			

Lane 8 Plasma 38

Band #	Rf	MgMk kDa	Peak RD	Trace RD x mm	Percent of Bands	Contour RD x mm²	Quantity	Band Name
8 - 2	0.141	195.02x	0.15	0.161	5.7			
8 - 3	0.165	163.13x	0.20	0.194	6.9			
8 - 5	0.205	121.33x	0.29	0.349	12.4			
8 - 7	0.273	82.58	0.37	1.429	50.6			
8 - 9	0.347	62.19	0.23	0.552	19.5			
8 - 15	0.521	36.69	0.14	0.140	5.0			

Lane 9 Plasma 55

Band #	Rf	MgMk kDa	Peak RD	Trace RD x mm	Percent of Bands	Contour RD x mm²	Quantity	Band Name
9 - 1	0.050	381.75x	0.14	0.763	40.8			
9 - 2	0.096	270.85x	0.13	0.043	2.3			
9 - 3	0.149	184.20x	0.19	0.229	12.2			
9 - 4	0.175	151.90x	0.19	0.141	7.5			
9 - 5	0.297	74.95	0.27	0.585	31.3			
9 - 6	0.529	35.85	0.11	0.109	5.8			

Lane 10 Plasma 87

Band #	Rf	MgMk kDa	Peak RD	Trace RD x mm	Percent of Bands	Contour RD x mm²	Quantity	Band Name
10 - 1	0.167	160.47x	0.39	0.679	9.0			
10 - 2	0.183	143.32x	0.45	2.682	35.6			
10 - 3	0.292	76.28	0.42	0.825	11.0			
10 - 4	0.310	71.37	0.38	0.422	5.6			
10 - 5	0.357	60.01	0.38	0.764	10.2			
10 - 6	0.424	48.75	0.39	1.490	19.8			
10 - 7	0.464	43.32	0.25	0.302	4.0			
10 - 8	0.487	40.57	0.15	0.245	3.3			
10 - 9	0.538	34.93	0.08	0.115	1.5			

SIGNED BY Shannon L Gleason **DATE** 9 Sept 2002

WITNESSED AND UNDERSTOOD BY _____ **DATE** 24 Oct 2002

CROSS REFERENCES:

Bayer HealthCare
Diagnostics Division

Self Testing Segment

Interoffice Memorandum

Date: July 7, 2003

Subject: Comparison of Uristatin Preparations

From: Shannon Gleason, Nancy C Leszczynski

To: Ron Sommer

cc: Howard Cooper

Project Name:	Uristatin	Date Assayed:	July 3, 2003
Project Number:	161200	Method of analysis:	Gel Electrophoresis
Sample Request No:	51566	Sample Analyte:	Purified Human Uristatin
Notebook Number:	RB27947		

Summary: Comparison of four lots of uristatin revealed unique protein banding patterns for each sample. While each lot contained the ~33 kDa band, lot 20-120 did not show the presence of the ~17 kDa band, and lot 124-11 did not show the ~63 kDa band.

Objective: The goal of this study was to compare two new Uristatin lots, 20-120 and 79-120, to three lots examined previously. However, the vial of Uristatin 80-117 was depleted, therefore comparison was only done against two of the old lots, 124-111 and 157-90.

Method: Samples were analyzed using a commercial pre-cast gel system (Invitrogen) NuPAGE 4-12% Bis-Tris with MES running buffer (reducing and non-reducing) following the manufacturer's recommended procedure. Samples were loaded at 2µg per lane. The assay was performed with a full set of standards, Mark12™ (Invitrogen) and SeeBlue®Plus2 (Invitrogen). Protein bands were stained with Colloidal Blue® (Invitrogen).

Results: The estimated molecular weights (MW) and percent composition for each band detected in the uristatin samples are shown in Table 1. The SDS-PAGE non-reducing and reducing gels are shown in Figures 1 and 2, respectively. Previously, analyses of uristatin preparations only included non-reducing gels as the electrophoretic separations were for Western blot studies.

Each uristatin lot demonstrates a slightly different protein banding pattern both on non-reducing and reducing gels. On the non-reducing gel, all lots contain the ~33 kDa protein band, while all but one lot (20-120) show significant amounts of the ~17 kDa band. In lots 124-111 and 157-90 the ~17 kDa band is the predominant component. In lots 20-120 and 79-120, the ~33 kDa protein band is the major component. In fact for this 2µg sample loading, lot 20-120 shows only a hint of staining in the ~17 kDa region. The ~63 kDa band is clearly present in uristatin lots 157-90, 20-120, and 79-120, but is not detectable in lot 124-111. Several additional bands are present in some of the uristatin preparations.

When the uristatin samples are examined under reducing conditions, all exhibit additional protein bands and some bands demonstrate a slight shift in estimated MW. Under these conditions, disulfide bonds are reduced thereby yielding more accurate MW estimations for the sample components. The 16-17 kDa band present in three lots of uristatin now migrates at 17 kDa for all samples. This may be due to slight differences in the method of preparation for lot 124-111 which could have left some secondary structure

in the 17 kDa band that caused it to migrate at a slightly lower MW (16 kDa). All the samples show an increase in the ~5 kDa band and in lot 124-111 this band now comprises about 1/3 of the protein.

	Non-Reducing		Reducing	
Uristatin	est. MW (kDa)	% of Bands	est. MW (kDa)	% of Bands
124-111	16	80	5	32
	33	15	17	24
	5	5	10	16
			15	12
			34	9
			8	8
157-90	17	87	17	64
	35	9	36	20
	63	4	15	7
			5	5
			11	3
			68	1
20-120	32	83	33	87
	63	10	73	5
	78	7	5	4
			90	3
			18	2
79-120	33	50	34	58
	17	25	17	24
	64	12	23	7
	21	11	73	5
	76	3	5	4
			44	2

Table 1. Estimated molecular weights and percent composition for each band on non-reducing and reducing gels. Proteins are listed in rank order from highest to lowest composition.

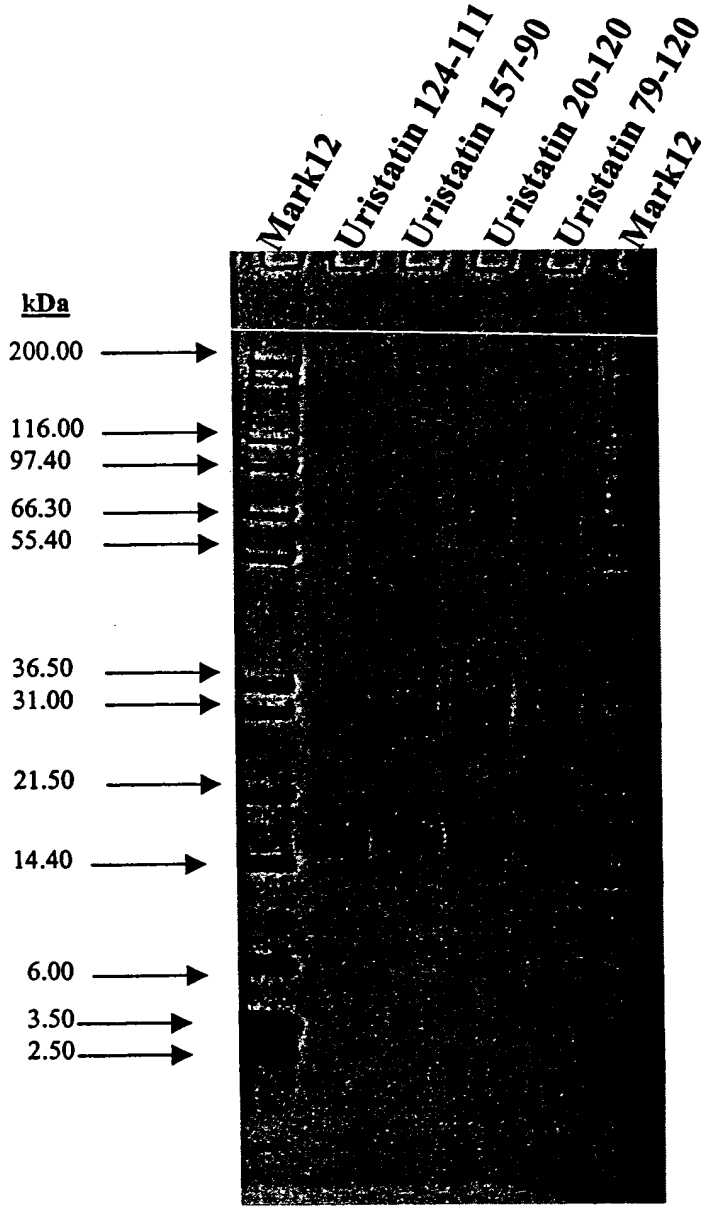


Figure 1. Uristatins: Non-Reducing Gel

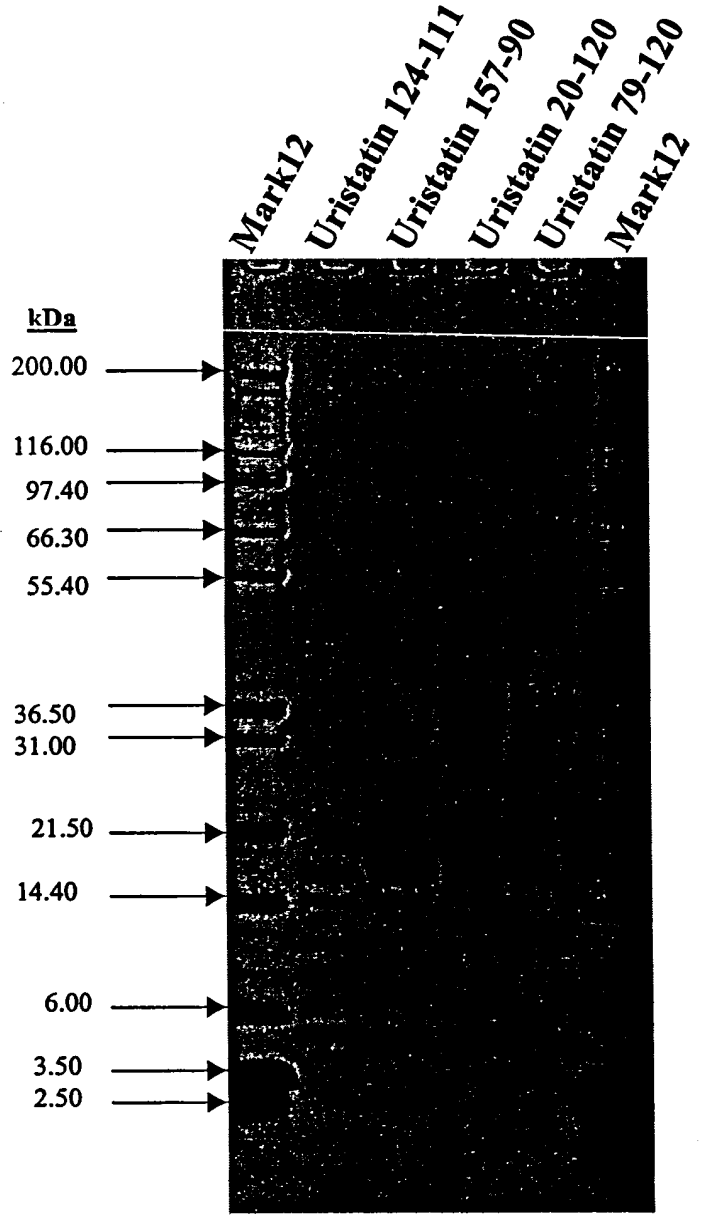


Figure 2. Uristatins: Reducing Gel

Bayer HealthCare
Diagnostics Division

Self Testing Segment

Interoffice Memorandum

Date: October 14, 2003

Subject: Comparison of Uristatin Preparations following storage

From: Nancy C Leszczynski, Shannon Gleason

To: Ron Sommer

cc: Howard Cooper, Mike Pugia, Linda Anderson, Solomon Murphy, Jim Profitt

Project Name:	Uristatin	Date Assayed:	October 8, 2003
Project Number:	161200	Method of analysis:	Gel Electrophoresis
Sample Request No:	51587	Sample Analyte:	Purified Human Uristatin
Notebook Number:	RB27947		

Summary: Comparison of four lots of Uristatin stored under three different conditions (lyophilized, -20°C and 4°C) revealed unique protein banding patterns for analyte each sample. Comparisons between the different storage conditions are shown in Table 1.

Note: While in some instances, additional bands did appear on the gel, they were not detected by the QS30 Optically Enhanced Densitometer System.

Objective: Previous SDS-PAGE analyses of several Uristatin samples have suggested a possible degradation of samples stored in the liquid state at 4°C. This analysis will look at samples that have been stored at either 4°C or –20°C after reconstitution of the lyophilized solids. In addition, the three lots of material submitted as lyophilized solid were reconstituted in 10mM PBS and were put under stability conditions of 4°C, -20°C and –70°C for 1, 2, 4 and 6 months.

Method: Samples were analyzed using a commercial pre-cast gel system (Invitrogen) NuPAGE 4-12% Bis-Tris with MES running buffer (reducing and non-reducing) following the manufacturer's recommended procedure. Samples were loaded at 2µg per lane. The assay was performed with a full set of standards, Mark12™ (Invitrogen) and SeeBlue®Plus2 (Invitrogen). Protein bands were stained with Colloidal Blue® (Invitrogen). Molecular weight estimations were determined using Mark12™ (Invitrogen). Densitometry data were generated using the QS30 Optically Enhanced Densitometer System. Images shown are pictures produced by the Eagle Eye II Still Video System as it generates better quality images. Images were cropped to eliminate SeeBlue®Plus2 (Invitrogen) markers. SeeBlue®Plus2 (Invitrogen) were used to track progress of the electrophoresis.

Results: Table 1 shows a simple comparison of the bands present for each lot under the various storage conditions. The reconstitution dates for the samples stored at 4°C were April 2002 for 124-111, and June 2003 for lots 79-120 and 20-120. The estimated molecular weights (MW) and percent composition for each band detected in the Uristatin samples are shown in Table 2. The SDS-PAGE non-reducing and reducing gels are shown in Figures 1 and 2, respectively. All samples stored at 4°C demonstrate a banding pattern that differs from the freshly reconstituted sample under one or both of the gel conditions. The newly initiated stability study will provide additional data when these changes occur.

doesn't say how much

Comparison of Bands present by Molecular Weight

124-111		
Reduced		
lyophilized	-20°C	4°C
35	34	
17	17	17
15	15	15
	11	10
		9
6	6	
5	5	5

124-111		
Non-Reduced		
lyophilized	-20°C	4°C
32	33	
16	16	16

lose 33 @ warmer conditions

20-120		
Reduced		
lyophilized	-20°C	4°C
89	89	
73	72	73
33	33	33
		22
		18
5	5	5

20-120		
Non-Reduced		
lyophilized	-20°C	4°C
		63
33		32
		17

Comparison of Bands present by Molecular Weight

157-90		
Reduced		
lyophilized		
72		
33		
18		
15		
7		
5		

157-90		
Non-Reduced		
lyophilized		
33		
18		

79-120		
Reduced		
	-20°C	4°C
	73	73
	34	35
	22	23
	17	18
	5	5

79-120		
Non-Reduced		
	-20°C	4°C
		63
		33
	17	17

Table 1. Comparison of bands present showing estimated molecular weights.

Estimated molecular weights and percent composition for each band

	Non-Reducing		Reducing	
Uristatin	est. MW (kDa)	% of Bands	est. MW (kDa)	% of Bands
124-111 lyophilized solid	16	97	17	49
	32	3	35	40
			5	5
			15	4
			6	3
20-120 lyophilized solid	33	100	33	93
			5	3
			73	3
			89	1
157-90 lyophilized solid	18	53	33	74
	34	47	19	19
			72	3
			15	3
			5	2.0
			7	0.3
124-111 -20°C	16	97	34	46
	33	3	17	38
			15	6
			5	6
			6	3
			11	1
20-120 -20°C			33	93
			5	3
			72	3
			88	1
79-120 -20°C	17	100	34	65
			17	27
			5	4
			73	3
			22	2

	Non-Reducing		Reducing	
Uristatin	est. MW (kDa)	% of Bands	est. MW (kDa)	% of Bands
124-111	17	100	5	41
4°C			11	19
			17	19
			9	10
			15	10
79-120	17	69	35	58
4°C	33	21	18	26
	63	10	5	8
			23	6
			73	2
20-120	32	88	33	89
4°C	64	10	5	6
	17	2	73	2
			18	2
			22	1

Table 2. Estimated molecular weights and percent composition for each band on non-reducing and reducing gels. Proteins are listed in rank order from highest to lowest composition.

51587NR-Uristatin

08OCT03

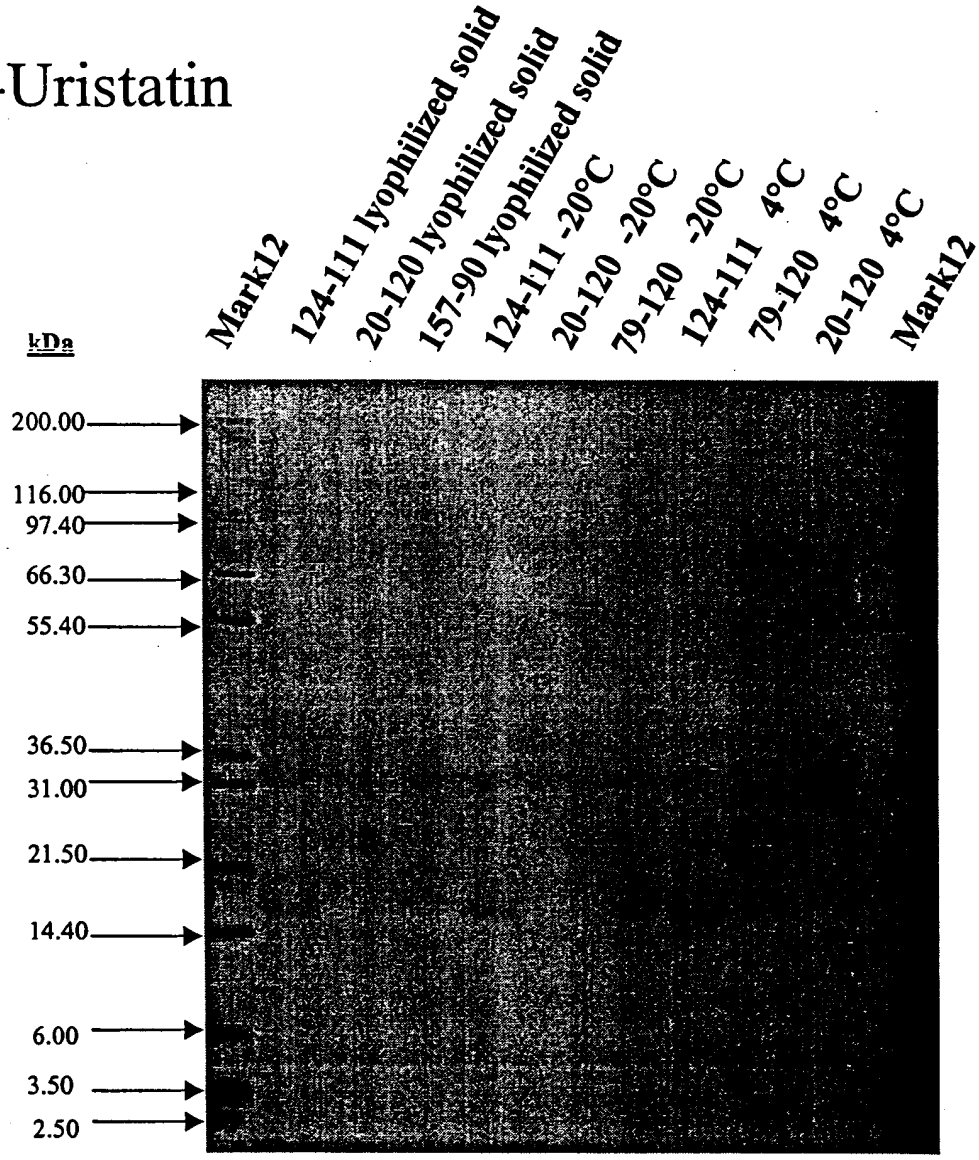


Figure 1. Uristatins: Non-Reducing Gel

51587R-Uristatin

08OCT03

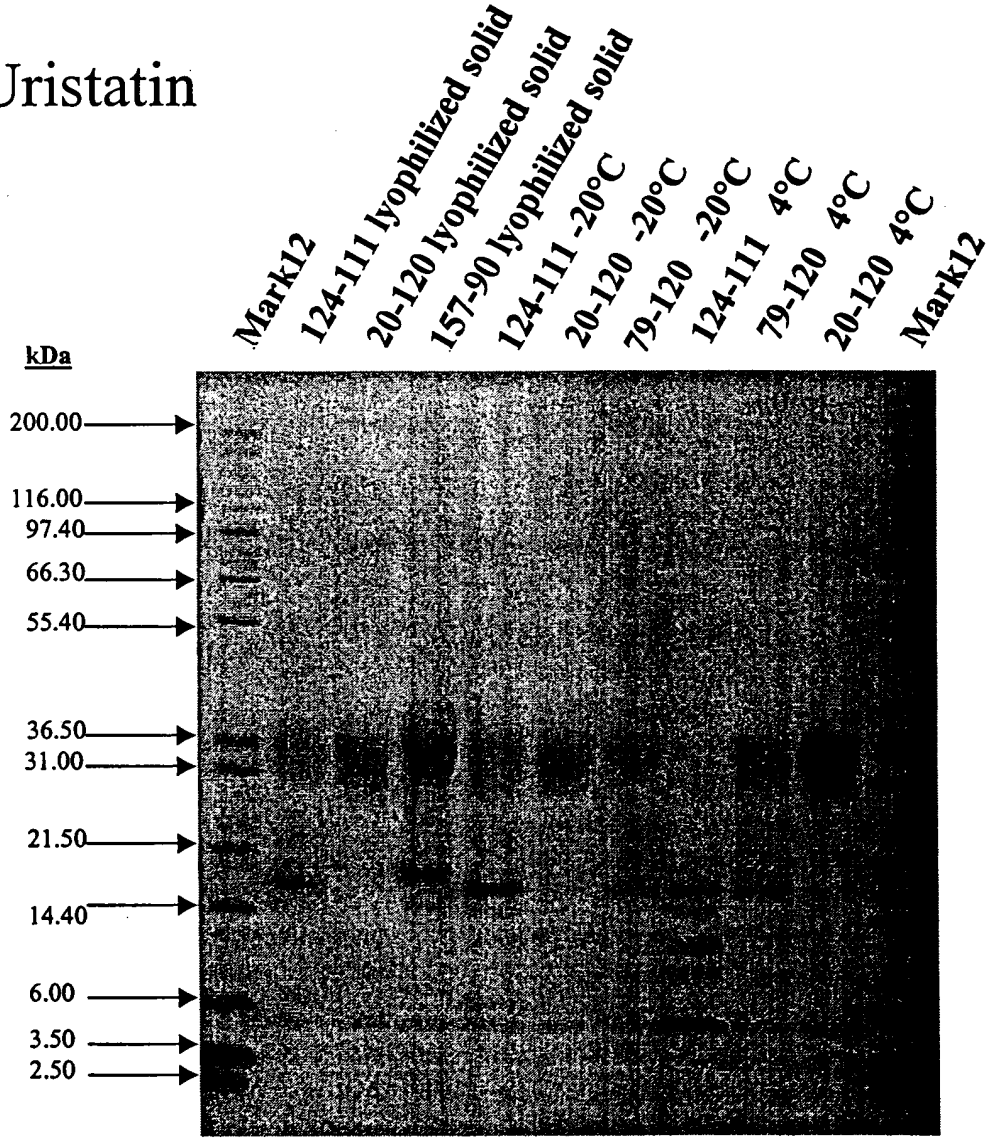


Figure 2. Uristatins: Reducing Gel

Complete content of each sample label:

Samples 1, 2 and 3 were lyophilized solid stored at 4°C then reconstituted.

Sample 1

Scipac
human Urinary Trypsin Inhibitor
Product Code:P205-2 Lot:124-111
Quantity:1mg Stor:2-8°C

Sample 2

Scipac
human Urinary Trypsin Inhibitor
Product Code:P205-2 Lot:20-120
Quantity:1mg Stor:2-8°C

Sample 3

Scipac
human Urinary Trypsin Inhibitor
Product Code:P205-2 Lot:157-90
Quantity:1mg Stor:2-8°C

Samples 4, 5 and 6 were frozen liquid, transferred without thawing, stored at -20°C

Sample 4

h UTI TBS 500µl
PC-P205-1 1mg/ml
lot-124-111 10/2002

Sample 5

20-120

Sample 6

79-120

Samples 7, 8 and 9 are liquid, stored at 4°C

Sample 7

Scipac
human Urinary Trypsin Inhibitor
Product Code:P205-1 Lot No:124-111
Quantity:1mg Stor:2-8°C

Sample 8

79-120

Sample 9

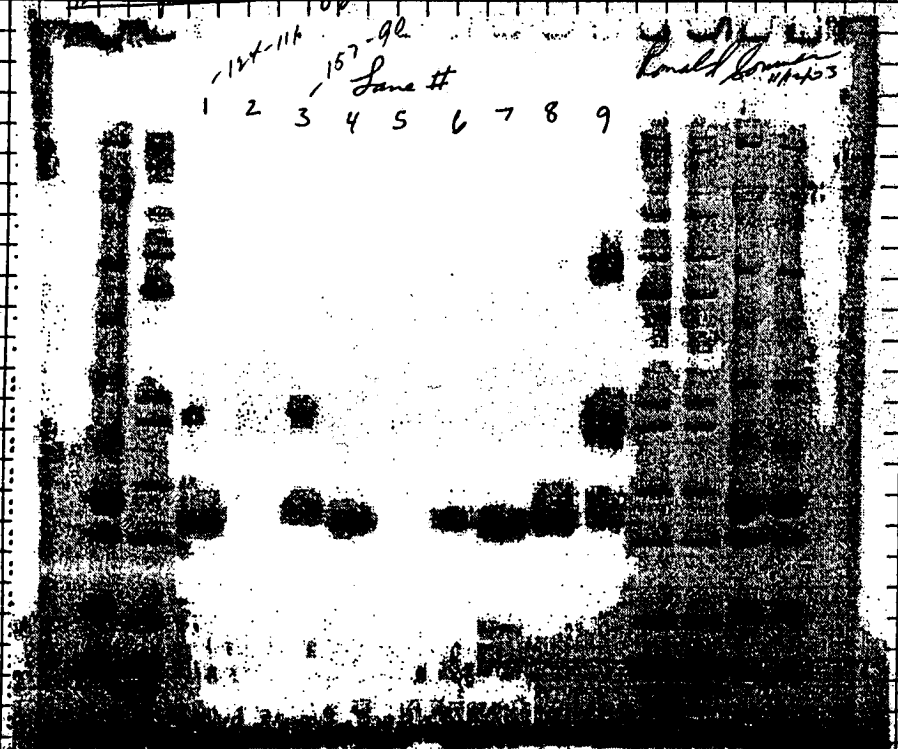
20-120

Note: Lyophilized samples reconstituted in 10mM PBS on 08OCT03. Stored at -20°C after reconstitution.
All samples (except stability aliquots) are being stored at -20°C after sampling.

BAYER CORPORATION

SUBJECT Re-analysis of gel bands for non-reducing gel from ARL Report #51587

I obtained the tiff files for the picture of the non-reducing gel from ARL report #51587 because the calculated values in that report did not agree with visual observation. I used PDA program (Jim Zimmerle) and an Excel spreadsheet that I prepared to analyze the area under the curve (K/S) for the jpg file generated in ARL Picture of non-reducing gel



SIGNED BY Ronald Sommer DATE Nov. 12, 2003

WITNESSED AND UNDERSTOOD BY [signature] DATE Feb. 26, 2004

CROSS REFERENCES: C:\my Documents\myfiles\YSCNTYT1\Variation\ARL\51587NRgel\YSNRgel_lane1.xls

Continued from page 32

Ronald Sommer 11/12/03

Lane 1

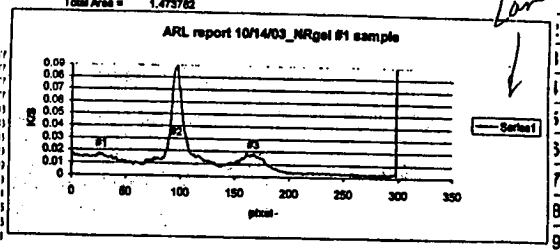
Lookup Table for Peak Area Calculations

Peak #	start pixel	start K/S	end pixel	End K/S	slope (m)	Intercept (b)
1	19	0.01499	46	0.01101	-0.000144444	0.0177344
2	67	0.00908	136	0.00627	-1.11268E-05	0.0008055
3	136	0.00827	201	0.00431	-6.25397E-06	0.0160006

#124-111 (lyophilized powder)

	Area	%Total
Area of peak #1 =	0.033343	2.3
Area of peak #2 =	1.145593	77.7
Area of peak #3 =	0.294847	20
Total Area =	1.473782	

%R?	K/S	peak #1 baseline K/S	peak #2 baseline K/S	peak #3 baseline K/S
83.35294	0.016824	0.01759	0.009784366	0.016837937
83.35294	0.016824	0.017446561	0.009783239	0.016775397
84.17647	0.014873	0.017301111	0.009772113	0.016712857
84.05882	0.015116	0.017158671	0.009760988	0.016650317
83.70588	0.015859	0.017012222	0.009749859	0.016587778
84.35294	0.014512	0.016867781	0.009738732	0.016525238
84.11765	0.014994	0.016723333	0.009727606	0.016462698
84.11765	0.014994	0.016578889	0.009716479	0.016400159
84.29412	0.014632	0.016434444	0.009705352	0.016337619
84	0.015238	0.01629	0.009694225	0.016275079
84.35294	0.014512	0.016145556	0.009683099	0.016212539
83.82353	0.015609	0.016001111	0.009671972	0.016150
84.17647	0.014873	0.015856667	0.009660846	0.016087461
84.11765	0.014994	0.015712222	0.009649718	0.016024921



Lane 2

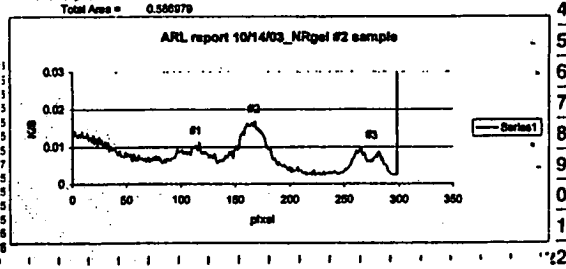
Lookup Table for Peak Area Calculations

Peak #	start pixel	start K/S	end pixel	End K/S	slope (m)	Intercept (b)
1	86	0.00569	136	0.00642	0.0000146	0.0044344
2	136	0.00642	217	0.00283	-4.67901E-05	0.0127835
3	248	0.00307	296	0.00241	-0.00001375	0.00646

#20-120 (lyophilized powder)

	Area	%Total
Area of peak #1 =	0.105320	17.9
Area of peak #2 =	0.324166	55.2
Area of peak #3 =	0.157492	26.8
Total Area =	0.586979	

%R?	K/S	peak #1 baseline K/S	peak #2 baseline K/S	peak #3 baseline K/S
85.23529	0.012788	0.004449	0.012736867	0.006468621
84.47059	0.014275	0.004483	0.012689877	0.006452
84.88236	0.013462	0.004478	0.012643886	0.006438
85.23529	0.012788	0.004492	0.012596896	0.006423
85.41177	0.012456	0.004507	0.012549906	0.006411
84.88235	0.013462	0.004522	0.012502716	0.006397
85.29412	0.012677	0.004536	0.012455926	0.006383
85.23529	0.012788	0.004551	0.012409136	0.00637
84.58823	0.01404	0.004565	0.012362348	0.006356
85.35294	0.012566	0.004580	0.012315556	0.006341
85.29412	0.012677	0.004595	0.012268766	0.006328
86.23529	0.012788	0.004609	0.012221975	0.006315
85.35294	0.012566	0.004624	0.012175185	0.006301
84.82353	0.013577	0.004638	0.012128396	0.006288



Lane 3

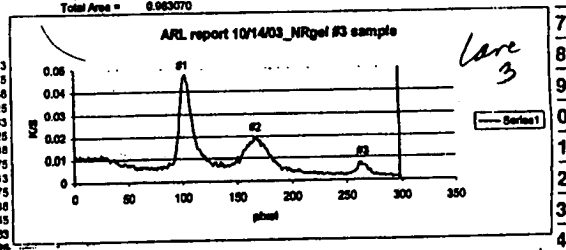
Lookup Table for Peak Area Calculations

Peak #	start pixel	start K/S	end pixel	End K/S	slope (m)	Intercept (b)
1	62	0.00649	140	0.00657	1.37931E-06	0.0063769
2	140	0.00657	213	0.0027	-3.93151E-05	0.0120741
3	252	0.00292	284	0.00119	-3.84375E-06	0.0126063

#157-90 (lyophilized powder)

	Area	%Total
Area of peak #1 =	0.592483	60.3
Area of peak #2 =	0.334853	34.1
Area of peak #3 =	0.055753	5.7
Total Area =	0.983070	

%R?	K/S	peak #1 baseline K/S	peak #2 baseline K/S	peak #3 baseline K/E
85.88235	0.011604	0.006378626	0.012034795	0.012567813
86.41177	0.010684	0.006379666	0.011995479	0.012529375
86.29412	0.010884	0.006381033	0.011956164	0.012490936
86.76471	0.010006	0.006382241	0.011916849	0.012452
85.94118	0.011499	0.006383579	0.011877534	0.012414063
86.64706	0.010269	0.006384517	0.011838219	0.012375625
86.17647	0.011067	0.006385855	0.011798904	0.012337188
86.41177	0.010684	0.006387193	0.011759589	0.01229375
86.17647	0.011067	0.006388931	0.011720274	0.012260313
86.58823	0.010367	0.006390669	0.011680959	0.012221875
86.70588	0.010192	0.006392207	0.011641644	0.012183438
86.17647	0.011067	0.006393345	0.011602329	0.012145
86.94118	0.011499	0.006394683	0.011563014	0.012106563
86.58823	0.010367	0.006396021	0.011523699	0.012068126



SIGNED BY Ronald Sommer DATE Nov. 12, 2003

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CROSS REFERENCES:

continued from page 33

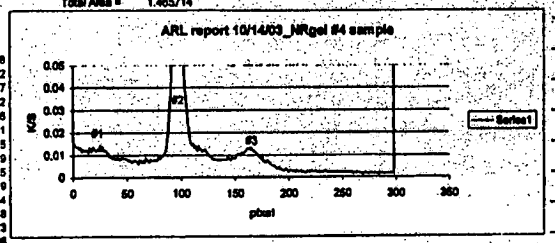
Lookup Table for Peak Area Calculations

Peak #	start pbal	start K/S	end pbal	End K/S	slope (m)	Intercept (b)
1	14	0.01099	43	0.00793	-0.000105517	0.0124672
2	74	0.00784	138	0.00801	2.65825E-06	0.0078434
3	139	0.00801	203	0.00277	-8.06154E-06	0.0191349

#124-111 (-20°C)

	Area of peak	%Total
Area of peak #1 =	0.048784	3.3
Area of peak #2 =	1.256569	85.7
Area of peak #3 =	0.160361	10.9
Total Area =	1.465714	

%R?	K/S	peak #1 baseline K/S	peak #2 baseline K/S	peak #3 baseline K/S
84	0.015238	0.01238172	0.007848094	0.019054308
84.47059	0.014276	0.01225621	0.00784875	0.018973692
84.70588	0.013807	0.01215069	0.007851406	0.018893077
84.78471	0.013692	0.01204517	0.007854063	0.018812462
84.70588	0.013807	0.01193966	0.007856719	0.018731846
85.29412	0.012677	0.01183414	0.007859375	0.018651231
84.88235	0.013462	0.01172862	0.007862031	0.018570615
85.82353	0.011708	0.0116231	0.007864688	0.01849
85.29412	0.012677	0.01151759	0.007867344	0.018409385
85.52941	0.012241	0.01141207	0.00787	0.018328769
85.52941	0.012241	0.01130655	0.007872656	0.018248154
85.58823	0.012134	0.01120103	0.007875313	0.018167538
85.64706	0.012027	0.01109552	0.007877969	0.018086923
85.23529	0.010989	0.01099	0.007880625	0.018006306



Lookup Table for Peak Area Calculations

Peak #	start pbal	start K/S	end pbal	End K/S	slope (m)	Intercept (b)
1	86	0.00495	136	0.00445	-0.00001	0.00561
2	136	0.00445	217	0.0018	-3.2716E-05	0.0088994
3	248	0.00215	296	0.0022	1.04167E-06	0.0018917

#20-120 (-20°C)

	Area of peak	%Total
Area of peak #1 =	0.091154	20.1
Area of peak #2 =	0.248605	54.4
Area of peak #3 =	0.115741	25.6
Total Area =	0.453500	

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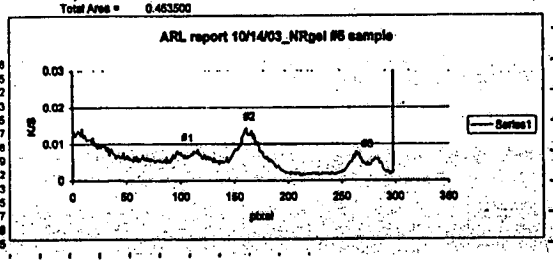
%R?	K/S	peak #1 baseline K/S	peak #2 baseline K/S	peak #3 baseline K/S
84.78471	0.013692	0.0056	0.008666667	0.001892708
85.52941	0.012241	0.00579	0.008833951	0.00189375
85.41177	0.012458	0.00578	0.008801235	0.001894792
85.17647	0.012899	0.00577	0.008768519	0.001895833
84.78471	0.013692	0.00576	0.008735802	0.001896875
84.88235	0.013462	0.00575	0.008703086	0.001897917
85.17647	0.012899	0.00574	0.00867037	0.001898958
85.47059	0.012349	0.00573	0.008637654	0.0019
84.47059	0.014276	0.00572	0.008604938	0.001901042
85.70588	0.01192	0.00571	0.008572222	0.001902083
85.36294	0.012568	0.0057	0.008539506	0.001903125
85.47059	0.012349	0.00569	0.00850679	0.001904167
85.17647	0.011087	0.00568	0.008474074	0.001905208
85.11765	0.011189	0.00567	0.008441358	0.00190625



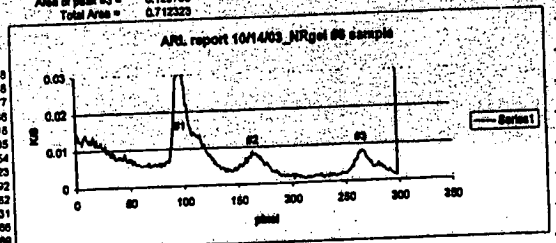
Lookup Table for Peak Area Calculations

Peak #	start pbal	start K/S	end pbal	End K/S	slope (m)	Intercept (b)
1	74	0.00562	146	0.00409	-0.00002125	0.0071925
2	146	0.00409	200	0.00215	-3.59259E-05	0.0093352
3	248	0.00215	296	0.00126	-1.87308E-06	0.0062658

#79-120 (-20°C)

	Area of peak	%Total
Area of peak #1 =	0.503438	70.7
Area of peak #2 =	0.085177	12
Area of peak #3 =	0.123706	17.4
Total Area =	0.712323	

%R?	K/S	peak #1 baseline K/S	peak #2 baseline K/S	peak #3 baseline K/S
84.29412	0.014632	0.00717126	0.009299259	0.006249038
84.64706	0.013923	0.00715	0.009263333	0.006232308
85.35294	0.012568	0.00712875	0.009227407	0.006215577
85.23529	0.012788	0.0071075	0.009191481	0.006198846
85.29412	0.012677	0.00708625	0.009155556	0.006182116
85.58235	0.011804	0.007065	0.00911963	0.006165385
84.94118	0.013349	0.00704375	0.009083704	0.006148654
84.88235	0.013462	0.0070225	0.009047778	0.006131923
84.41177	0.014393	0.00700125	0.009011852	0.006115192
85.05882	0.013123	0.00698	0.008975926	0.006098462
85.17647	0.012899	0.00695876	0.00894	0.006081731
85.64706	0.012027	0.0069375	0.008904074	0.006065
85.17647	0.012899	0.00691625	0.008868148	0.006048269
85.76471	0.011814	0.006906	0.008832222	0.006031538



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CROSS REFERENCES:

continued from page 34

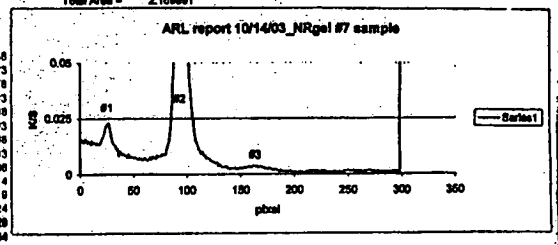
Lookup Table for Peak Area Calculations

Peak #	start pbal	start K/S	end pbal	End K/S	slope (m)	Intercept (b)
1	19	0.0139	39	0.30906	-0.000237	0.018303
2	66	0.00743	145	0.30268	-0.000059378	0.0112894
3	146	0.00269	204	0.30081	-3.16949E-05	0.0072758

#124-111 (4°C)

	Area of peak	%Total
Area of peak #1 =	0.080958	3.7
Area of peak #2 =	2.054274	94.7
Area of peak #3 =	0.034661	1.6
Total Area =	2.169891	

%R?	K/S	peak #1 baseline K/S	peak #2 baseline K/S	peak #3 baseline K/S
84.05882	0.015116	0.018066	0.01123	0.007144068
84	0.015238	0.017829	0.011170625	0.007112373
84.52941	0.014167	0.017592	0.011111125	0.007180678
84.41177	0.014393	0.017355	0.011051875	0.007148983
83.70588	0.015859	0.017118	0.0109925	0.007117288
83.94118	0.015361	0.016881	0.010933125	0.007085593
84.64706	0.013923	0.016644	0.010873125	0.007185898
84.29412	0.014632	0.016407	0.010814375	0.007122203
84.35294	0.014512	0.01617	0.010755	0.006990508
84.94118	0.013349	0.015933	0.010695625	0.006958814
84.17647	0.014873	0.015696	0.01063625	0.006927119
84.78471	0.013692	0.015459	0.010576875	0.006895424
84.41177	0.014393	0.015222	0.0105175	0.006863729
84.70588	0.013807	0.014985	0.010458125	0.006832034



Lookup Table for Peak Area Calculations

Peak #	start pbal	start K/S	end pbal	End K/S	slope (m)	Intercept (b)
1	67	0.00711	134	0.00596	-1.86857E-05	0.00824
2	134	0.00596	190	0.00184	-7.39286E-05	0.0158884
3	241	0.00446	287	0.00118	-0.00005	0.0165

#79-120 (4°C)

	Area of peak	%Total
Area of peak #1 =	0.954275	71.1
Area of peak #2 =	0.194165	14.5
Area of peak #3 =	0.193679	14.4
Total Area =	1.342139	

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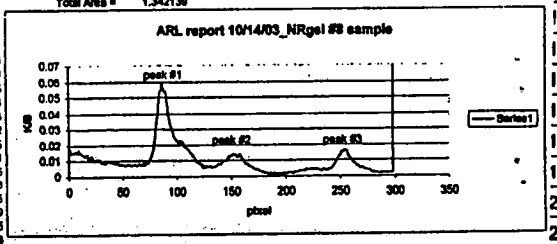
%R?	K/S	peak #1 baseline K/S	peak #2 baseline K/S	peak #3 baseline K/S
83	0.01741	0.00822313	0.0158125	0.01346
84.41177	0.014393	0.00820627	0.015735571	0.0164
84.23529	0.014762	0.0081894	0.015664643	0.01336
84.17647	0.014873	0.00817254	0.015593714	0.0163
84.41177	0.014393	0.00815567	0.015616786	0.01626
83.70588	0.015859	0.00813881	0.015442857	0.0162
84	0.015238	0.00812194	0.015368929	0.01815
84.35294	0.014512	0.00810507	0.015295	0.0161
83.29412	0.016753	0.00808821	0.015221071	0.0160
84.23529	0.014762	0.00807134	0.015147143	0.016
84.29412	0.014632	0.00805448	0.015073214	0.0159
84.64706	0.013923	0.00803761	0.014999286	0.0159
84.70588	0.013807	0.00802075	0.014925357	0.01585
85	0.013236	0.00800388	0.014851429	0.0158



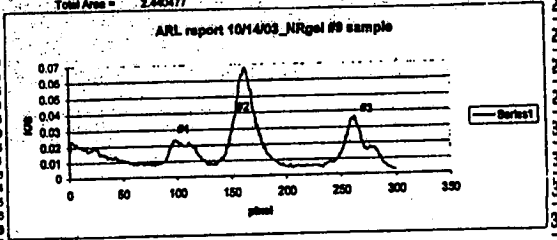
Lookup Table for Peak Area Calculations

Peak #	start pbal	start K/S	end pbal	End K/S	slope (m)	Intercept (b)
1	79	0.00776	134	0.00751	-4.54545E-06	0.0081191
2	134	0.00761	206	0.00556	-2.84865E-05	0.0110592
3	235	0.0058	299	0.00297	-6.98438E-05	0.0208633

#20-120 (4°C)

	Area of peak	%Total
Area of peak #1 =	0.411851	16.9
Area of peak #2 =	1.342517	55
Area of peak #3 =	0.686308	28.1
Total Area =	2.440477	

%R?	K/S	peak #1 baseline K/S	peak #2 baseline K/S	peak #3 baseline K/S
83	0.01741	0.00811455	0.011032703	0.020601438
83.47059	0.023696	0.00811	0.011006216	0.020743594
80.52941	0.023536	0.00810545	0.01097973	0.02063375
80.76471	0.022906	0.00810091	0.010953243	0.020623906
81.23529	0.021672	0.00809636	0.010926757	0.020564063
81.47059	0.021071	0.00809182	0.01090027	0.0205C4219
81.11765	0.021977	0.00808727	0.010873784	0.020444375
81.58823	0.020775	0.00808273	0.010847297	0.020314531
81.76471	0.020334	0.00807818	0.010820811	0.020214688
81.82353	0.020189	0.00807364	0.010794324	0.020214844
81.70588	0.02048	0.00806909	0.010767838	0.0:0205
82.29412	0.019047	0.00806455	0.010741351	0.0201-5156
82.35294	0.018909	0.00806	0.010714865	0.020015313
82.17647	0.019329	0.00805546	0.010688378	0.0200 15469



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SIGNED BY Ronald Sommer DATE Nov. 12, 2003

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CROSS REFERENCES:

Bayer HealthCare
Diagnostics Division
Self Testing Segment

Interoffice Memorandum

Date: March 29, 2004

Subject: Comparison of Uristatin Preparations following 24 and 16-weeks of storage

From: Nancy C Leszczynski

To: Ron Sommer

cc: Shannon Gleason, Howard Cooper, Mike Pugia, Linda Anderson, Solomon Murphy, Jim Profitt

Project Name:	Uristatin	Date Assayed:	March 24, 2004
Project Number:	16100	Method of analysis:	Gel Electrophoresis
Sample Request No:	51587	Sample Analyte:	Purified Human Uristatin
Notebook Number:	RB27998		

Summary: 24 and 16 week stability checkpoint comparison of four lots of Uristatin stored under three different conditions (4°C, -20°C and -70°C) revealed unique protein banding patterns for each sample lot.

Objective: Previous SDS-PAGE analyses of several Uristatin samples have suggested a possible degradation of samples stored in the liquid state at 4°C. This analysis will look at samples that have been stored at 4°C, –20°C and -70°C after reconstitution of the lyophilized solids. Three lots were reconstituted to 1mg/mL in 10mM PBS on October 8, 2003 and were put under stability conditions of 4°C, -20°C and –70°C for 1, 2, 4 and 6 months. This is the 24-week stability checkpoint.
An additional sample was submitted for analysis after the 1 month stability check was completed; Uristatin lot #93-90 was reconstituted to 1mg/mL on December 3, 2003 in 10mM PBS and stored at stability conditions of 4°C, -20°C, and -70°C. The initial analysis date of 93-90 was December 3rd. This is the 16-week checkpoint. This is the last stability checkpoint for this study.

Method: Samples were analyzed using a commercial pre-cast gel system (Invitrogen) NuPAGE 4-12% Bis-Tris with MES running buffer (reducing and non-reducing) following the manufacturer's recommended procedure. Samples were loaded at 5µg per lane. The assay was performed with a full set of standards, Mark12™ (Invitrogen) and SeeBlue®Plus2 (Invitrogen). Protein bands were stained with Colloidal Blue® (Invitrogen). Molecular weight estimations were determined using Mark12™ (Invitrogen).
Densitometry data were generated using the Kodak Image Station 2000R and analyzed using Kodak 1D Image Analysis Software. This system replaces the QS30 Optically Enhanced Densitometer System, used for previous analyses.

Conclusion: Uristatin Lot 124-111 shows a shift in the relative percentage towards the 17 kDa band at 4°C. Uristatin 157-90 continues to show a shift towards the 18 kDa band at the 4°C temperature. 20-120

appears to be stable at 24 week 4°C temperature, however a slight increase in the 17 kDa band is starting to appear. All samples stored at –20 and -70°C remain stable. 93-90 shows an increase in the 17 kDa band after storage at 4°C for 16 weeks.

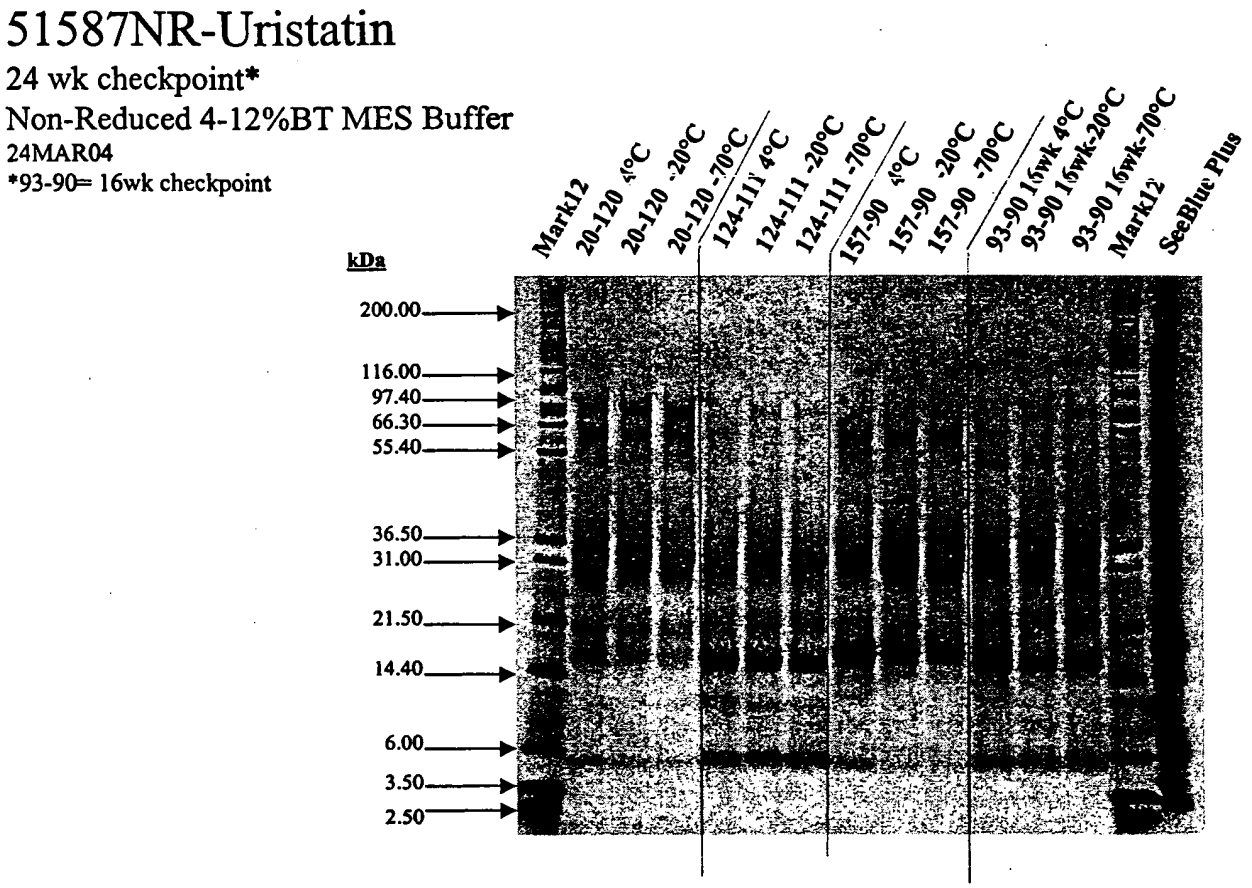

Figure 1. Uristatins: Non-Reducing Gel

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